Abstract

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Title of Doctoral Thesis: The development of fast and efficient UHPLC-MS/MS method for the determination of salicylic acid and its derivatives

The aim of this thesis was to develop a rapid method for qualitative and quantitative analysis of salicylic acid (SAL) and its derivatives, 2,3- and 2,5-digydroxybenzois acids (DHBAs) using ultra high performance liquid chromatography (UHPLC) coupled with tandem mass spectrometry (MS/MS).

This indirect method can be used to assess hydroxyl radical formation. Due to its high reactivity and a very short lifetime, direct evidence of presence of free radicals has low sensitivity and it is difficult. In this method, hydroxyl radical adducts are measured. Salicylic acid is used as a hydroxyl radical trap, that produces stable products, 2,3- a 2,5-dihydroxybenzoic acids, which can be identified and quantified. These compounds can be used as a sensitive indicator to measure hydroxyl radical formation both in vitro and in vivo.

Liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) offers considerable advantages: speed, selectivity and sensitivity.

Two chromatographic columns were tested. The first, Acquity BEH Shield RP18 provided insufficient results. The second, Acquity BEH C18, provided sufficient retention and selectivity for the separation of all analytes.

The best separation was achieved when a sample volume of 5µl was used and the mobile phase was 0,1% formic acid and methanol 70:30 (isocratic flow). Limit of detection was 0.54 ng/ml (2,5-DHBA), 0.37 ng/ml (2,3-DHBA) and 0.36 ng/ml (SAL). Limit of quantification was 1.78 ng/ml (2,5-DHBA), 1.21 ng/ml (2,3-DHBA) and 1,18 ng/ml (SAL).

Keywords: Salicylic acid, 2,3-dihydroxybenzoic acid, 2,5-dihydroxybenzoic acid, UHPLC-MS/MS, free radicals, iron, Fenton reaction